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STUDIES IN THE MORPHOLOGY OF MALARIAL PLASMODIA AFTER THE ADMINISTRATION OF QUININE, AND IN INTRACORPUSCULAR CONJUGATION.*†

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IN reviewing the literature upon the action of quinine on the malarial plasmodia, one is surprised to find how much confusion apparently exists as to the exact effect of this drug on the parasites, and, therefore, the most effective period for its administration in the treatment of the malarial fevers. Indeed, we find so great an authority as Manson[1] saying, in the latest edition of his work upon tropical diseases:

> In what way quinine acts has not been satisfactorily explained. Some, reasoning from the toxic influence this drug exerts upon many kinds of free amoebae, say that it acts in malaria in the same way; that is, as a direct poison to the parasite. . . . . Others maintain that it acts in stimulating the phagocytes, the natural enemies of the parasites. Some experimentalists allege, on the other hand, that it paralyses the white corpuscles. It is said by some to be most effective against the free spores and the very young intracorpuscular forms, but inoperative against the more mature parasites; hence they advocate giving it early in the parasitic cycle. Others, on the contrary, maintain that it is operative only on the large intracorpuscular forms, and therefore advocate its use at a late stage of the cycle.

This statement, coming from so distinguished an authority upon malaria, well illustrates the uncertainty that exists regarding the action of quinine upon the malarial plasmodia, despite the fact that the drug is known to be a specific in malarial disease, and that it is comparatively easy to determine the exact morphological changes produced by it in the plasmodia.

I have devoted much study to this subject and have examined many cases of malaria after the administration of quinine with a view to determining the exact morphological changes produced by

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[1] *Tropical Diseases*, 4th ed., New York, 1908.

the drug in the various species of plasmodia, and the present contribution gives the results of this study, together with data concerning the process of intracorpuscular conjugation which I have collected since I described this phenomenon in December, 1905. The material for these studies has embraced infections with all of the species of human plasmodia, the infections being in the persons of soldiers of the army stationed in the Philippines and returning therefrom, as well as in native Filipinos. My method has been to examine the blood at regular intervals after the administration of quinine, studying both fresh and stained preparations, and in this way to observe the effect of the drug upon every stage of the life-cycle of the plasmodia in man; in some instances the drug was given in divided doses at regular intervals, while in others it was administered just before the expected paroxysm or at various stages of the development of the plasmodia. In every instance it was found that more pronounced results were obtained when the quinine was administered in divided doses, at regular intervals.

The material embraced in the study of intracorpuscular conjugation comprises many hundred cases of malarial infection, including infections with every species of human plasmodium, and the results are such that I am firmly of the opinion that this process is of great importance in the life-history of the plasmodia and that it explains most logically the occurrence of latent infection and recurrence in malarial disease. Certain it is, that the process is too frequently observed to be considered as accidental and that it is entirely distinct from the double and triple infection of corpuscles frequently observed, especially in estivo-autumnal infections.

The confusion existing regarding the action of quinine upon the plasmodia is, no doubt, largely due to imperfect staining methods employed by various investigators, and to the fact that more attention has been paid to the appearance of the parasites in the fresh blood than to their staining reactions. In my work I have employed the staining method of Wright, modified slightly as described in previous contributions.[1] I have found this method very satisfactory, as it is easy of application and uniform in its results. By it, the chromatin of the nucleus of the parasite stains a brilliant red, the vesicular

[1] Craig, *Amer. Med.*, 1905, 10, pp. 982, 1029; *Jour. Infect. Dis.*, 1908 5, p. 324.

portion of the nucleus remains unstained, while the protoplasm stains a robin's-egg blue.

PART I.

THE CHANGES IN THE MORPHOLOGY OF THE MALARIAL PLASMODIA PRODUCED BY QUININE.

In considering the morphological changes produced in the malarial plasmodia by quinine, it is necessary to describe those occurring in the forms concerned in schizogony, and also those occurring in the forms of the plasmodia developed in man which are concerned in sporogony. But before giving my results a brief summary of the results of the work of other observers may be of interest:

In 1867, Binz[1] found that solutions of quinine, when brought in contact with some of the infusoria, produce morphological changes ending in the death of the organisms experimented with, and from this fact drew the conclusion that the beneficial action of the drug in malarial infections was due to the destruction of the plasmodia. In 1881, Laveran[2] demonstrated that a 1:1000 solution of quinine produced immediate cessation of the movements of the plasmodia when it was added to blood containing them, and his results were soon confirmed by other observers.

As regards Plasmodium vivax (the tertian plasmodium), Golgi[3] claimed that quinine produced very decided effects, even the adult plasmodia being markedly changed in appearance. This work was done upon fresh blood specimens and he found that the drug produced a shrinkage in the parasites and a granular degeneration of the protoplasm, while the segmenting bodies were found to have a smaller number of segments. Romanowsky,[4] using his method of staining, found that in the young parasites the vesicular portion of the nucleus disappeared, and that the chromatin stained less intensely. In the older parasites he found that the protoplasm stained poorly; that the chromatin was reduced in amount and stained less intensely; and that the vesicular portion of the nucleus disappeared. Mannaberg[5] studied both fresh and stained specimens of the tertian plasmodium, finding that quinine produced in the former lessened ameboid motion and that several hours after the administration of the drug the parasites underwent fragmentation. He claimed that in stained preparations the chromatin entirely lost its power of staining, and that most of the segmenting bodies were devoid of chromatin. On the other hand, Ziemann found that when the drug is given during apyrexia the chromatin stained as usual, altho fragmentation of the parasites occurred.

The later observations of Schaudinn[6] are of interest in that he studied preparations stained by the Romanowsky method, in which the plasmodia were present in every stage of their human life-cycle. Unfortunately he studied only one case of double tertian infection, so far as can be judged by his paper, and it is therefore hardly justifiable to consider his results as conclusive for quartan or estivo-autumnal infections, or even for tertian infections as a class. Schaudinn found that quinine rendered the young tertian schizont less stainable and deformed in appearance; the nucleus, as well as the protoplasm, was fragmented, altho the nuclear chromatin still stained. In the older schizonts fragmentation of both the protoplasm and nucleus was very common, while both stained poorly. He found no certain evidences of the action of the drug upon the reproductive phases of the plasmodia, except that the merozoites stained diffusely and the nucleus appeared broken and scattered. He found that quinine affected the developing sexual forms in the same manner as the schizonts, but that the fully developed gametes were resistant to its action.

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[1] *Archiv f. path. Anat.*, 1867, 41, p. 23.

[2] *Compt rend. Acad. Sci.*, 93, p 627.

[3] *Deut. med. Wchnschr.*, 1892, 18, pp. 613, 685, 707, 729.

[4] *St. Petersburg med. Wchnschr.*, 1891, Nos. 34, 35.

[5] *Die Malarial Krankheiten*, Nothnagle, Philadelphia, 1905.

[6] *Arb. a. d. kais. Gesundh.*, 1902, 19, p. 169.

As regards Plasmodium malariae (the quartan plasmodium), the observations are not as numerous and are conflicting. Antolisei[1] could not find any morphological changes in this plasmodium as the result of quinine, and his results were confirmed by Golgi, who claimed that the drug had no effect upon the adult quartan plasmodium. Mannaberg found the same changes occurring in this organism after quinine administration as were described by him in the tertian parasite.

The observations concerning the action of quinine upon the estivo-autumnal plasmodia (Plasmodium falciparum and Plasmodium falciparum quotidianum) are still more conflicting. Baccelli[2] found that the administration of the drug, while producing an increase in the ameboid activity, did not produce any degenerative changes; Marchiafava and Bignami[3] claimed that the drug caused the young parasites to leave the infected cells but that no morphological changes occurred, with the exception that pigment formation was hindered, thus proving that the drug interfered with the nutrition of the organism. La Monaco and Panichi[4] state that the effect of the drug varies with the strength of the solution employed; weak solutions causing swelling and increased ameboid activity; strong solutions greatly increasing ameboid activity; and that very strong solutions caused shrinkage and death. Their work was done upon fresh specimens, the quinine solution being added to the blood after it had been removed from the body.

From this brief summary it is evident that the results of various observers are conflicting and the reason for this is not always easily apparent. As regards the method of experimentation by adding quinine solutions to blood containing the parasites after the latter have been removed from the body, it is obvious that it is an imperfect one and one from which conclusions should not be drawn as regards the effect of the drug when it is circulating in the blood. Cappogrossi[5] has shown that the exit of the plasmodia from the red corpuscles, so often observed when a solution of quinine is added to infected blood, is not due to the specific action of the drug, but is purely physical, being due to the hypotonicity of the solution. He found that an isotonic salt solution containing quinine does not cause the extrusion of the parasites from the red corpuscles but reduces their motility and renders their protoplasm more granular.

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[1] *Riforma Med.*, 1890, 6, p. 68.

[2] *Studien über Malaria*, Berlin, 1895.

[3] "Malaria," *20th Century Practice*, New York, 1900.

[4] *Untersuchungen zur Naturlehre des Menschen und der Tiere*, 1901, 17, p. 22.

[5] *Il Policlinico*, 1901.

In detailing the results of my studies upon this subject I shall describe the changes produced by quinine upon all of the species of plasmodia occurring in man, as shown in both fresh and stained preparations of the blood, considering each species separately.

Plasmodium vivax (tertian plasmodium).—This species is most easily studied because of its comparatively large size and the ease with which the details of its morphology may be recognized. The changes produced by quinine in this species are very marked and are evident in the living organism as well as in stained preparations.

Fresh preparations.—Quinine produces marked morphological changes in this organism during every stage of its life-cycle in man, with the possible exception of that stage just preceding sporulation and the fully developed gametes. The action is most marked upon the young, rapidly growing plasmodia and least upon the fully developed forms. In the growing forms a great reduction is observed in the amount of pigment which is developed, thus proving that the drug influences very markedly the nutrition of the parasite. This is in accord with the observations of Marchiafava and Bignami. If quinine be administered at the time of sporulation it undoubtedly kills many of the merozoites at once, but many escape and undergo development, to a greater or less extent, within the red corpuscles. Death may occur at any stage of this development and the changes produced in the morphology of the parasite do not differ from those usually observed when the drug is given after the organisms have entered the erythrocytes. Those parasites, however, which survive and sporulate, generally present atypical sporulating forms, thus proving that the drug has influenced their development, even tho it has not destroyed them.

If the drug be administered, in a single tertian infection, at the time that the blood contains the young intracorpuscular plasmodia, the first change observed in the living parasite is a great increase in the ameboid activity, followed, after an hour or so, by a marked decrease. The ameboid activity is often so greatly stimulated that it is extremely difficult to follow the movements of the pseudopodia of the organisms, which are projected and withdrawn so rapidly as to make it almost impossible to keep them in focus. The "ring-form," so frequently observed in this type of infection, is lost during the period of stimulation, but as the motility becomes lessened it is regained, and after the cessation of motion the parasite is generally spherical or "ring-shaped," and more sharply outlined than is the normal organism.

The protoplasm becomes more granular in appearance, and while, in a case untreated with quinine, in which the parasites are in a similar developmental stage, a few granules of pigment are usually observed, pigment is never found in the plasmodia presenting the phenomena I have just described.

If the drug be continued and the blood be examined at regular intervals, it will be noticed that it acts upon the plasmodia in every stage of development up to the time of sporulation. Many of the organisms perish, for it is noticed that the number present in the blood specimens gradually decreases as the time for sporulation approaches, but some of the parasites mature and sporulate, altho nearly always presenting marked morphological changes, degenerative in character.

In the early pigmented stage of the parasite quinine produces the same initial stimulation of ameboid activity, followed, in many instances, by complete cessation of motion, and eventually, by fragmentation. After the formation of pigment quinine appears to produce fragmentation more frequently than while the parasite is in the hyaline stage of growth, and fragmented parasites are very numerous in the blood from tertian infections, if the blood be examined upon the fever-free day. The increase in the refractive index and the granular appearance of the protoplasm of the pigmented parasites is especially noticeable in those organisms which do not fragment but continue their development.

Examined during the afebrile period, the blood will be found to contain numerous erythrocytes which present within them plasmodia which are divided into several distinct portions, devoid of motion. These are fragmented parasites and should be carefully distinguished from those parasites in which ameboid motility is so pronounced as to cause the extrusion of numerous pseudopodia simultaneously, and their appearance at various portions of the infected cell. Many of the fragments contain pigment, which may be sluggishly motile. Sometimes the fragmented organisms appear to be extruded from the red corpuscle and may be observed lying free between the blood cells. The protoplasm of the fragments is generally more refractive than that of the normal plasmodium and much more granular in appearance. After liberation from the erythrocyte the fragments soon become very granular and finally disintegrate. It is very common in tertian infections, under treatment with quinine, to observe free plasmodia in almost every stage of development, presenting the degenerative changes mentioned. Such plasmodia are always more or less fragmented, the fragments frequently being held together by a delicate filament of protoplasm. The pigment within the fragments may remain motile for hours if the specimen of blood be kept upon a warm stage, but eventually the fragments separate, become mere masses of granular material, and disintegrate. The fragmentation due to quinine occurs at every stage of the human life-cycle of the tertian plasmodium up to the presegmenting stage, but at this stage and in the sporulating stage I have never observed any evidence of this form of degeneration.

While fragmentation terminates the career of many of the plasmodia which have escaped the action of quinine during the earlier period of development, some of the plasmodia continue to develop and go on to sporulation. Upon such parasites, up to the presegmenting stage, quinine, if given in one large dose, has a marked effect, causing shrinkage and granular deposits in the protoplasm; an increased refractive index of this substance; and a decrease in the amount of pigment developed during further growth. Some of the parasites are killed but the majority sporulate. It is rare to observe fragmentation in the more fully developed plasmodia, and it is very seldom observed in organisms which entirely fill the red corpuscles.

If the quinine has acted upon the plasmodia during their developmental cycle, as it does when it is administered in divided doses, the effects produced are similar in kind to those just described, but a smaller number of the parasites are able to sporulate, having been so injured during their earlier stages as to perish in large numbers as sporulation approaches. Many of the organisms which do sporulate show a lessened number of merozoites and many of these are found to be sterile when stained specimens are examined.

So far as I have been able to determine, quinine produces no morphological changes in the presegmenting or segmenting parasites when it is administered at the time these stages are present in the blood. While this is so, it is evident that it destroys many of the merozoites after sporulation has occurred, as shown by the small number of these which can be demonstrated in the erythrocytes some hours after sporulation.

The morphological changes observed in living specimens of Plasmodium vivax, which are produced by quinine, may be summed up as follows:

a) An initial stimulation of ameboid activity, followed by a decrease in activity, and eventually in cessation of motion.

b) A granular degeneration of the protoplasm.

c) An increased refractive index of the protoplasm.

d) Fragmentation of the parasite, followed by the apparent extrusion of the fragmented organism from the red corpuscle.

e) A marked decrease in the amount of pigment developed in those parasites which undergo development during the administration of the drug. I believe that the extrusion of the fragmented parasites is only apparent, the real explanation of the process being that they are liberated by the death and disintegration of the infected erythrocyte. I have never observed the active extrusion of a malarial plasmodium from the red corpuscle and do not believe that it occurs as the result of the action of quinine.

The changes produced in the gametes are similar to those described in the forms of the human life-cycle but the drug affects the gametes only in the early stages of their development. The fully developed tertian gamete shows no morphological changes after the administration of quinine and I have repeatedly observed flagellation after the drug has been administered for several days. If, however, the quinine be administered early in the infection, the number of gametes is greatly reduced, many of them being destroyed during the early stages of their development.

Stained preparations.—While the morphological changes produced by quinine are easily observed in living tertian plasmodia, they are much better demonstrated in specimens stained with Wright's method, as in such preparations the chromatin of the nucleus is rendered visible and we are thus able to trace the action of the drug upon this essential portion of the parasite. My observations are confirmatory of those of

Romanowsky and Schaudinn in some particulars but I have been unable to demonstrate that the chromatin loses its staining power, as claimed by Romanowsky, or that it stains less intensely, as claimed by Schaudinn. I have found, on the contrary, that the chromatin stains more intensely after the administration of quinine than before, but that the quality of the staining is markedly affected. The brilliant red stain of the chromatin observed in normal specimens of plasmodia is replaced by a very dark purple or almost black color, indicating that some important change has occurred in the chemical nature of this substance. The unstained vesicular portion of the nucleus is found to have disappeared, and the chromatin is often arranged irregularly in the protoplasm in the young plasmodia, thus indicating that the nucleus has been broken up and the chromatin liberated. The claim made by Ziemann that the chromatin stains normally and does not become fragmented at any stage of the development of the parasite I have not been able to confirm. If the drug be given just before sporulation no change in the appearance of the chromatin is noticed but if the drug be administered early in the intracorpuscular stage of development, the markedly atypical division of the chromatin is very noticeable. The plasmodia which develop into sporulating bodies after the administration of quinine almost invariably present atypical conditions in stained preparations, the merozoites staining irregularly and being reduced in number, while many of them are devoid of chromatin.

The changes produced by quinine are best studied in stained preparations from patients to whom the drug has been given at three-hour intervals, the blood being examined at intervals of three or four hours. In this way it is not difficult to study the effect of the drug upon every stage of development of the tertian plasmodium as observed in the blood of man. The administration of the drug in a single large dose produces similar morphological changes, tho these are not so pronounced in character.

The effect of quinine upon the unpigmented schizonts, while still in the "ring-stage," is evidenced in stained preparations by the deeper staining of the protoplasm, the very dark violet or almost black staining of the chromatin, and the absence of the unstained area which normally surrounds the latter. The protoplasm takes a deep blue color, this substance in the normal plasmodium taking a lighter blue stain. The brilliant crimson color of the chromatin normally observed is replaced by a dark violet or almost black color, this change in staining reaction being always present in the young schizonts if the drug be administered just before or at the time of sporulation of the plasmodia. I have never observed fragmentation of either the protoplasm or nucleus at this stage of development, but minute irregularities in the contour of the "rings" are not infrequently noted, and very rarely the chromatin presents slight evidences of fragmentation, minute granules being present about the periphery of the large chromatin mass.

In organisms a little farther advanced in development the changes produced by the drug are similar to those just described but the stimulation of ameboid motion is shown in the very bizarre shape of the parasites. Fragmentation is not infrequently observed even in the young schizont after it has lost its "ring-form" and before the development of pigment. In such instances the infected erythrocyte contains two or more irregular masses of protoplasm stained a deep blue color while the chromatin may or may not be fragmented at this early stage. If the chromatin be fragmented it may be observed distributed in the fragments of protoplasm but where fragmentation has not occurred it is either contained in one of the detached portions of protoplasm or lies free within the erythrocyte. Rarely the extrusion of the chromatin is observed at this stage of development. The chromatin takes a deep violet or almost black color. In most of the plasmodia the vesicular portion of the nucleus cannot be demonstrated.

The effect of the drug is most marked after the development of pigment and when the plasmodium is from one-half to three-quarters grown. In such plasmodia the evidence of fragmentation is most prominent and the changes in the chromatin most easily demonstrated.

Many of the erythrocytes contain fragmented plasmodia, the fragments being irregular in shape and staining a deep blue or almost purple color. Pigment may be present in them but the decrease in the amount of the latter as compared to the amount in normal plasmodia at the same stage of development is most striking.

The chromatin stains a dark violet or almost black color and there is little evidence of increase in the amount or of division of this substance. While in normal plasmodia at similar stages of development the chromatin has increased notably in amount and has divided into fine granules and filaments arranged loosely in one portion of the organism, in the plasmodia affected by quinine the chromatin has increased slightly, if at all, in amount, and is arranged in either one large clump or in two or three smaller ones; it is never observed in the form of small granules or threads in parasites showing other evidences of the action of the drug. The chromatin may be situated in one or more of the fragments of protoplasm or lie free within the erythrocyte, generally near the periphery. The extrusion of the chromatin from the plasmodium is quite frequently observed at this stage of development. The vesicular portion of the nucleus of the plasmodium is not visible. It is evident from the morphological appearances described that these fragmented plasmodia have been destroyed by the drug but others are observed which present no evidences of fragmentation but which are undoubtedly injured, though to a less degree, by the drug. These plasmodia are irregular in shape; the protoplasm takes a deeper blue stain than normally; while the chromatin is collected in one or two large clumps near the periphery of the organism. Evidence of normal division of the chromatin is almost lacking, and in some instances, extrusion of this substance has occurred, thus proving that quinine possesses the power of rendering the plasmodia sterile. The pigment is markedly decreased in amount and generally arranged in irregular clumps near the periphery of the plasmodium. The organisms, which show little evidence of ameboid activity, are smaller than normal and the deeper blue staining of the protoplasm is probably due to a concentration of the latter. In the unfragmented plasmodia there is no evidence of the vesicular portion of the nucleus.

Extracellular plasmodia undergoing fragmentation, or isolated fragments of such plasmodia, are frequently observed at this time in stained preparations. The fragments stain a deep blue and may be devoid of pigment or filled with it, while rarely a clump of chromatin may be observed within a fragment. The occurrence of these extracellular parasites brings up the question of their origin. Are they extruded from the infected red corpuscle or are they liberated by the disintegration of the latter? The evidence pointing to their extrusion is slight and inconclusive and I believe that they are liberated by the disintegration of the infected erythrocyte. That quinine produces degeneration of the infected red corpuscle is evidenced by the occurrence of polychromatophilia and basophilia after its administration to a much greater extent than before.

If quinine has only been administered for a day or two the preparations will show plasmodia normal in appearance, but they are few in number in comparison with the injured plasmodia. The plasmodia which are able to resist the action of quinine until fully developed present strong evidence of the inhibitory action of the drug upon normal schizogony. Even at this late stage some of the plasmodia are found to be undergoing fragmentation but the majority are evidently in the presegmenting stage. In the latter the protoplasm stains a distinct blue, the organism appears shrunken, and the pigment is small in amount and collected in large granules or clumps about the periphery of the parasite. The chromatin has increased but little in amount, stains a dark violet, and is arranged in two or three irregular masses within the protoplasm. The small amount of chromatin and its arrangement are eloquent of the action of quinine in preventing typical sporulation, for in the normal plasmodia at this stage of development the chromatin has increased enormously in amount, stains a brilliant crimson, and is distributed in numerous small clumps throughout the protoplasm.

Extracellular fragmented plasmodia are still present but are not nearly so numerous as in earlier stages of development of the parasites. Indeed, fragmentation due to quinine is most frequent in the early pigmented stages of development and this may be said to be the characteristic form of degeneration when the parasites are from one-half to three-quarters developed.

In those plasmodia which are undoubtedly preparing to segment, the protoplasm stains a diffuse blue, the lines of cleavage of the developing merozoites being indistinct or irregular. The chromatin is very small in amount as compared with normal plasmodia, and is collected in the protoplasm in very irregular masses varying much in size and situated at irregular intervals within the protoplasm. Some plasmodia show only one or two such masses of chromatin while others may contain several, but they are always atypical in appearance and stain a very dark violet instead of the brilliant crimson color observed in normal plasmodia. The pigment is small in amount and generally arranged about the periphery of the plasmodium.

The sporulating plasmodia, which have partially resisted the action of quinine during their development, present morphological changes of great interest. The number of merozoites is generally reduced, some of the plasmodia showing only six or eight; the contour of many of them is irregular, and many appear devoid of chromatin, consisting of protoplasm only. The merozoites which contain chromatin are often irregular in shape, and in most of them there is no trace of the unstained portion of the nucleus, while the chromatin is atypical in appearance, staining an almost black color, and consisting of two or more granules instead of the single large dot so characteristic of the normal merozoite. Parasites are not infrequently observed undergoing sporulation in which only two or three of the merozoites contain chromatin, the remainder being simply masses of blue-stained protoplasm, irregular in shape, and sometimes much vacuolated. In some of the plasmodia at this stage of development a process of partial sporulation appears to have occurred, a portion of the organism consisting of well-defined merozoites, while the remainder consists of a mass of protoplasm in which no trace of division into merozoites can be demonstrated. Such organisms forcibly suggest the bodies described by Schaudinn as macrogametes undergoing parthenogenesis. I have never observed such parasites in the blood of patients who have not taken quinine.

The merozoites which are devoid of chromatin are generally irregular in shape and stain a deep blue or bluish-green. A few granules of residual pigment may sometimes be observed within them, indicating a fragmentation of the plasmodium before sporulation is completed. In fact, I believe that most of the merozoites devoid of chromatin originate from plasmodia which were in an almost perishing condition when sporulation occurred. If this be the true explanation of the phenomenon, it is evident that quinine possesses the power of injuring every developmental stage in the human life-cycle of Plasmodium vivax.

Normal sporulating plasmodia may be observed in small number in the blood of patients who have not been treated with quinine for more than a day or two, along with those presenting the morphological changes described.

The morphological changes produced in the young gametes are similar to those produced in the schizonts, and, for this reason, do not need to be further described. There are the same changes in the staining reaction of the protoplasm and chromatin; the same lack of evidence of increase in the amount of the latter; and the same absence of the vesicular portion of the nucleus. The gametes are apparently not affected by the drug after they have developed much pigment and in preparations from patients who have suffered for some time from the infection, gametes normal in appearance may be observed along with numerous full-grown or sporulating plasmodia presenting the most profound evidence of the action of the drug.

The morphological changes produced in Plasmodium vivax by quinine, as shown in stained specimens, may be summarized as follows:

1. Changes in the staining reaction of the protoplasm and chromatin.
2. Fragmentation of both the protoplasm and chromatin.
3. Prevention of normal increase in the amount of chromatin and of normal division.
4. The production of atypical sporulating plasmodia, in which many of the merozoites appear devoid of chromatin.
5. Similar changes in the young gametes as regards the protoplasm and chromatin.

Plasmodium malariae.—The changes produced in Plasmodium malariae by quinine are very similar to those already described as occurring in Plasmodium vivax as regards morphology and I cannot confirm the observations of Antolisei that the older quartan plasmodia are not affected by this drug. While there is some slight evidence that this species is more resistant to the action of quinine than is Plasmodium vivax, it is nevertheless true that the drug acts upon every stage of schizogony.

Fresh preparations.—In the young "ring-forms" marked increase in ameboid motion is noticed after the administration of the drug, succeeded, as in Plasmodium vivax, by decreased motility, and finally, by cessation of all motion. In the young pigmented forms fragmentation is often observed and ameboid activity is absent. The pigment is small in amount and immotile. The half and three-quarters grown organisms present a very granular protoplasm, fragmentation is common, and the pigment is reduced in amount. In the presegmenting parasites the reduction in the amount of pigment is very noticeable, while the distribution of this substance in the protoplasm is irregular and atypical. The sporulating parasites show a reduced number of merozoites and the latter appear granular and very refractive.

Stained preparations.—Practically the same changes are observed as occur in Plasmodium vivax. The protoplasm stains a deep blue and the chromatin takes a dark violet color. The latter often appears fragmented and the amount present is much smaller than normal in all stages of development of the parasite. Fragmentation of the protoplasm and extrusion of the chromatin are frequently noticed and even in the adult parasite fragmentation is far from being rare. The vesicular portion of the nucleus is absent from the pigmented organisms and the amount of pigment is reduced. In the presegmenting parasites the chromatin is small in amount and arranged in two to three irregular clumps near the periphery of the plasmodium. Extracellular fragmenting plasmodia are frequently observed and appear as do those of Plasmodium vivax.

The sporulating quartan plasmodium stains diffusely blue after administration of quinine; the number of merozoites is reduced; while in many organisms there occurs an irregular mass of protoplasm which shows no trace of division and is devoid of chromatin, along with a few atypical merozoites. The merozoites are irregular in contour and are often devoid of chromatin, while the protoplasm stains a deep blue color.

As in tertian infections, apparently normal plasmodia are observed along with the degenerative forms, provided the quinine has not been administered for more than three or four days.

The effect of quinine upon the gametes of Plasmodium malariae is similar in every respect to its effect upon the gametes of Plasmodium vivax, the young gametes only being acted upon by the drug, so far as can be ascertained.

Plasmodium falciparum and Plasmodium falciparum quotidianum.—The effect of quinine upon the estivo-autumnal plasmodia is the same in both species, for which reason they will be considered together. I have studied the action of the drug upon every stage in the human life-cycle of these plasmodia with the exception of the fully developed pigmented organisms and the sporulating stage. As is well known, the latter forms are very seldom observed in the peripheral blood, it being necessary to puncture the spleen in order to obtain material for study, and because of this my observations have been so limited as regards these stages in the development of the estivo-autumnal plasmodia that I do not feel justified in drawing any definite conclusions from them. In one fatal case of pernicious tertian estivo-autumnal malaria to which quinine had been administered some hours before death, the splenic smears showed many sporulating plasmodia, some of which presented a smaller number of merozoites than normal, while the morphological changes were similar to those described as occurring in Plasmodium vivax and Plasmodium malariae.

Fresh preparations.—In preparations of fresh blood the estivo-autumnal plasmodia while in the "ring-stage" appear more refractive after the administration of quinine than normally, while the ameboid activity is greatly stimulated. The "ring-form" is often lost, the parasites becoming discoid in shape, as described by Marchiafava and Bignami, or the contour becomes irregular so that they resemble the young hyaline stage of the benign tertian plasmodium. I have never observed the extrusion of the plasmodia from the erythrocyte which has been described by Marchiafava and Bignami as characteristic of the action of quinine upon these species of plasmodia. The increased ameboid activity of the plasmodia causes them frequently to change their position in the infected corpuscle, and they sometimes move very rapidly from one portion of the red cell to another. After a variable time the movements become slower and finally cease.

The protoplasm of the plasmodia appears more granular than normal, while the outline of the parasite, when motion has ceased, is very sharply defined. Upon the pigmented "rings" and the larger pigmented bodies observed in the peripheral blood, quinine appears to produce a granular degeneration of the protoplasm and a great increase in the refractive index of the plasmodia. Ameboid motion is absent and the outline of the organism is very sharply cut. The amount of pigment is apparently normal.

Stained preparations.—That quinine produces marked morphological changes in the estivo-autumnal plasmodia is amply shown by the study of stained preparations of blood from patients to whom this drug has been administered. Marchiafava and Bignami concluded from their studies that no marked morphological changes occurred in these plasmodia after the administration of quinine, while pigment formation ceased after such administration. I cannot confirm their conclusions, for I have found that quinine produces changes in the morphology of these plasmodia similar to those occurring in Plasmodium vivax and Plasmodium malariae and that pigmented plasmodia are often observed in the blood for several days after the administration of the drug.

The protoplasm of the "ring-forms" stains more intensely blue than normally while the chromatin stains a very dark violet color. The unstained area surrounding the chromatin is generally absent and the young plasmodium often consists of a stained mass of protoplasm, oval or round in shape, containing a small clump of chromatin. Many of the plasmodia in this early stage of development are irregular in form, due to the stimulation in ameboid activity. Extrusion of the chromatin from the plasmodia is rarely observed.

In the larger pigmented plasmodia the morphological changes produced by quinine, as shown in stained preparations, are similar in every respect to those observed in Plasmodium vivax and Plasmodium malariae, except that fragmentation is less common and extracellular forms are generally absent. I do not feel justified in describing the changes produced in the sporulating organisms, as my material has been so limited since I began the study of this subject. In the one case I have already mentioned that the morphological changes observed were identical with those occurring in the other species of human plasmodia, altho more normal plasmodia were observed.

I have not been able to observe that the administration of quinine produces any morphological changes in the fully developed estivo-autumnal gametes (crescents) but the drug affects the early stages of development of this type of the plasmodia in the same manner as it does the young gametes of the other species of plasmodia.

The summary given of the changes produced by quinine in Plasmodium vivax serves equally well for Plasmodium falciparum and Plasmodium falciparum quotidianum, so far as I have been able to determine, with the exception that in the estivo-autumnal plasmodia the drug less frequently causes fragmentation.

Practical deductions.—It is evident from the observations noted that quinine, whenever given, is effective in destroying the plasmodia, but it is also evident that the drug is most effective if given in divided doses at regular intervals, thus keeping the blood charged with it. Given in this manner the plasmodia are continually exposed to the action of the drug and the morphological changes observed in the plasmodia prove that the drug is capable of destroying them at every stage in their life-cycle in man, with the possible exception of the sporulating stage, but even in this stage it produced an atypical division of the parasite, resulting in a lessened number of merozoites and sterility of many of the latter.

If the drug be administered in one large dose just before sporulation very many of the merozoites are at once destroyed but those which are able to survive for a few hours develop and sporulate in a normal manner, the drug having been excreted before the cycle of development has been completed, some stages escaping entirely from the effect of the drug. If, on the other hand, after a moderate-sized initial dose, the drug be continued at intervals of three or four hours, those parasites which have escaped the first dose are not free to develop normally but are continually exposed to the action of the drug, which is always present in the blood.

My observations show that the plasmodia under such conditions are injured during every stage of their growth, many perishing before sporulation, while those that sporulate do so in an atypical manner. The conclusions drawn regarding the time of the administration of quinine, as the result of the study of the morphological changes produced by the drug, are not only justified theoretically, but have the confirmation of actual clinical experience.

I have the clinical records of over 2,000 patients suffering from the various types of malarial fever, in which quinine was given in divided doses at regular intervals. In all of these cases recovery from the infection was prompt and more rapid than in similar cases in which the drug was administered in one large dose prior to, or immediately following, sporulation. In the tertian infections a second chill very rarely followed the administration of the drug, altho most of the cases showed a rise of temperature upon the day of the expected paroxysm. In only about 20 per cent of these, however, did the temperature reach 101° F., and in most of them it did not rise above 100° F. In the quartan infections, a second paroxysm never occurred if the drug was administered in this way, provided the initial dose was given within 12 hours after the first paroxysm. In the estivo-autumnal infections the method of administering quinine in divided doses, at regular intervals, is the only one which should be employed, for it is generally impossible to ascertain the exact time of the expected paroxysm in most of these infections, so that it is impossible to give the drug in one large dose, in the expectation of destroying the merozoites before they infect the erythrocytes. In the many hundred cases of estivo-autumnal malaria in which I have seen quinine administered in divided doses, the results have been most satisfactory, provided the treatment was begun before the appearance of pernicious symptoms, and even in the pernicious cases, the best results are secured by giving the drug hypodermically or intravenously, in divided doses. In ordinary cases of estivo-autumnal malaria the administration of the drug in divided doses results in a fall of the temperature to normal within three days, and in the milder infections the fever generally disappears within two days. In estivo-autumnal infections quinine should never be administered in one large dose during the 24 hours, as such treatment invariably results in the febrile condition persisting for several days, during which time pernicious symptoms may develop. I have observed very serious results follow the administration of quinine in this manner, some of the patients so treated dying from pernicious attacks which might have been controlled had the drug been administered in divided doses at regular intervals.

PART II.

STUDIES UPON INTRACORPUSCULAR CONJUGATION IN THE MALARIAL PLASMODIA.

In previous communications[1] I have described a process of conjugation observed in the malarial plasmodia, occurring within the infected erythrocytes, and apparently of importance in the life-history of the parasites, as it occurs very frequently and is uniform in its phenomena.

This process was first described, so far as I have been able to ascertain, by Mannaberg,[2] who considered that it was the first stage in the development of the crescent form of the estivo-autumnal plasmodia; while later, the same phenomena were described by Ewing,[3] but were considered by him as of rare occurrence and of little importance in the life-cycle of the plasmodia.

Mannaberg described the process very accurately. He says, regarding the method of reproduction of the malarial plasmodia: "The reproduction of the malarial parasites takes place through a simple segmentation (spore formation) in the developed condition, probably also by the formation of spores after previous conjugation and encystment." He describes the occurrence within the erythrocytes of two or more plasmodia and distinguishes multiple infection of the red corpuscles from the true conjugation forms. Regarding the latter he says: "These paired parasites occupy usually the edge, less frequently the center of the blood corpuscle. In some pairs the protoplasmic membranes are clearly seen lying close to one another; at other times they are hazy or hardly to be recognized, so that in the latter case one gets the impression that through coalescence the two parasites have produced a new form, which in its structure betrays its origin." Again he says: "Returning now to the paired ameboid malarial parasites, the analogy of the zoölogical facts just mentioned must lead us to the opinion that we have possibly here to do with a process of conjugation." Mannaberg gave this subject much attention and was able to observe actual blending of the two plasmodia with the production of a new and larger organism. Regarding this observation he states: "I gave, during this last summer, very careful attention to fresh preparations, especially to the double invasion of the red blood corpuscles, viewing them for long periods, in order to see whether one could observe the process of coalescence of two parasites. My efforts were successful, for I was able repeatedly to see how two parasites coalesced and formed a larger body. After this there can be no doubt that the ameboid parasites, coalescing with each other, can from larger bodies."

The observations of Mannaberg are of great interest and value, for while he erred in interpreting conjugation as preliminary to the formation of the crescents, or gametes, he gives the first accurate description of the process and observed it in the living organisms, as well as in stained preparations.

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[1] Craig, *Amer. Med.*, 1905, 10, pp. 982, 1029; *Jour. Infect. Dis.*, 1907, 4, p. 10; *Inter. Clinics*, 1907, 3, p. 273; *Philippine Jour. Sci.*, 1906, 1, p. 523.

[2] "The Malarial Parasites," *New Sydenhem Soc.*, 1894, p. 241.

[3] *Clinical Pathology of the Blood*, New York, 1903, p. 454.

Mannaberg's observations were made upon estivo-autumnal plasmodia, while those of Ewing were made upon both the tertian and estivo-autumnal plasmodia. The latter observer described the process as occurring but rarely in these infections and speaks of conjugation between both the young plasmodia and larger, pigmented forms. His description of the process, in so far as it concerns the young, unpigmented plasmodia, is excellent, but it is evident that he confused multiple infection of the erythrocyte with conjugation, for true conjugation occurs in the hyaline plasmodia only before the formation of pigment, and the large pigmented tertian conjugation forms described by him were undoubtedly instances of the multiple infection of the red cell by two tertian plasmodia which had developed pigment. Ewing was undecided regarding the significance of the process in the malarial plasmodia, regarding which he says: "The extent and significance of this form of conjugation it is difficult to determine. In the cases in which it can be profitably studied, parasites are very abundant, and in most cases few indications of the process can be detected. It is, therefore, probably not essential to sporulation, and when parasites are scanty the chances of finding typical examples of conjugation pairs are greatly reduced, but the peripheral blood may not be a complete index of the process in the internal viscera. It seems probable that conjugation occurs in the first generation of the infection, and becomes less frequent as the disease progresses, the infection in the human host thereby tending to limit itself."

It is interesting to observe that Ewing believed that the process has something to do with the limitation of the infection, altho he evidently did not regard it as a means by which the infection is kept alive within man.

For over seven years, in the course of other studies upon the malarial plasmodia, I have observed the occurrence of this form of conjugation in the plasmodia of man and have carefully studied its various stages. The process occurs in every malarial infection in which quinine has not been given early; it occurs in recurrent attacks in which acute symptoms have been present for several days; and in it I believe that we have the most rational explanation of latency and recurrence in malarial disease. The material for my studies upon this subject has embraced over 500 cases of malarial fever observed in this country in soldiers returning from the Philippine Islands, and nearly 200 cases observed in the Philippines. Of these, 175 were latent infections (infections without symptoms), about 200 were acute initial infections, and the remainder recurrent infections, one or more paroxysms having occurred days or weeks before the patient came under my observation.

Conjugation and the species of plasmodium.—Intracorpuscular conjugation occurs in all of the species of malarial plasmodia infecting man but is most frequently observed, and most easily studied, in infections with the estivo-autumnal plasmodia, the reason being the greater resistance of these species to treatment and, therefore, the longer duration of the infection. There is no reason to believe, however, that the process is less frequent in infections with Plasmodium vivax and Plasmodium malariae, which have had no treatment, altho it is less frequently observed. This form of conjugation is a normal phase of the life-cycle of all species of human malarial plasmodia under certain conditions, but, as will be seen later, it does not occur in infections of slight duration, or in infections in which proper treatment has been initiated early in the course of the disease.

Time of occurrence.—Intracorpuscular conjugation never occurs during the first few days of an acute malarial infection and for this reason it is not observed in initial acute malarial attacks, or in recurrent cases in which quinine has been administered promptly. I have never observed the process in cases in which but two or three paroxysms had occurred prior to treatment, but after the infection has persisted for a week or more, intracorpuscular conjugation is invariably observed. An exception to the statement as regards quinine is found, of course, when the dose of that drug is insufficient, and it is because of the resistance of the estivo-autumnal plasmodia to quinine that we so frequently observe this form of conjugation even in cases which have been treated for short periods of time. In all acute and recurrent malarial infections, which have persisted for a week or more, instances of intracorpuscular conjugation will be found, if carefully searched for, in the peripheral blood. In latent infections the process is rarely observed in the peripheral blood, and only in long-continued infections.

The process of intracorpuscular conjugation is most frequently observed in infections which have lasted for a long time, and in which there have been several recurrences. Thus in estivo-autumnal infections, especially those presenting pernicious symptoms, the process is almost always observed, as these infections are generally of long duration and are sure to recur unless very thoroughly treated with quinine. In all infections in which sporulation has occurred several times, and where the plasmodia are very numerous, intracorpuscular conjugation is observed. In those pernicious cases in which the symptoms develop suddenly during a short initial attack of the fever, this form of conjugation is never observed, so far as I have been able to determine. As regards the time of occurrence of intracorpuscular conjugation we may state that it occurs only after the plasmodia have multiplied in the usual manner for several generations, and thus it is observed only in those malarial infections which have persisted for some time. The process is evidently intended to preserve the race of plasmodia from extinction as the result of repeated multiplication. In one case of fatal pernicious malaria due to the tertian estivo-autumnal plasmodium, in which the parasites had multiplied to such an extent that almost every other red corpuscle was infected, examples of intracorpuscular conjugation were observed in practically every microscopic field.

Multiple infection of the erythrocyte and intracorpuscular conjugation.—Multiple infection of the erythrocyte by the malarial plasmodia is of very frequent occurrence, especially in infections with Plasmodium falciparum quotidianum, and should be carefully distinguished from intracorpuscular conjugation. Cropper[1] describes multiple infection of the erythrocyte in a case of pernicious malaria and states that when such multiple infections occur the plasmodia develop as usual and go on to sporulation. Cropper thinks that this observation precludes the occurrence of the form of conjugation described in this paper, but if he had read Mannaberg's paper, and my original description of this form of conjugation, it would have been apparent to him that we had not confused conjugation with multiple infection, and that it would be impossible to mistake the two phenomena. In my original description of intracorpuscular conjugation I say:[2] "As is well known, it is very common in specimens of blood from patients suffering from estivo-autumnal infections to see cells containing two or more "ring-forms" of the plasmodia: such parasites, however, are not always conjugating forms." Again, in the same contribution, I say: "We must admit that in some instances two parasites develop independently, so far as we can determine, for I have seen red corpuscles containing two plasmodia at different stages of development, and this is not uncommonly observed in tertian infections."

From these quotations it is evident that I did not confuse multiple infection of the erythrocyte with intracorpuscular conjugation, as

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[1] *Proc. Soc. Trop. Med.*, 1907–1908, 1, p. 145.

[2] Craig, *Amer. Med.*, 1905, 10, pp. 982, 1029.

Cropper appears to believe. Multiple infection bears no resemblance whatever to intracorpuscular conjugation, as will be appreciated when we consider the morphological phenomena of the latter, and it would be impossible for anyone who has studied the malarial plasmodia concerned in this process to confuse it with such infection.

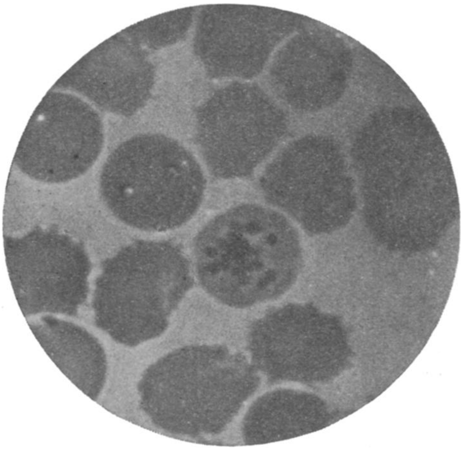

FIG. 1.—Blood smear from case of estivo-autumnal malaria, showing multiple infection of the red cell with two "ring-forms" and a sporulating plasmodium. Note the small size of the "rings" and the minute dot of chromatin.*

In multiple infection, while the parasites may lie in apposition, there is no evidence of the union of the protoplasm and nucleus, and, as has been known for years, the two organisms, if they survive, develop in a normal manner and eventually sporulate. In intracorpuscular conjugation there is a visible union of the two plasmodia and the production of a single and larger organism. Mannaberg was the first to demonstrate this fact in living plasmodia, and I have been able to confirm it repeatedly. In addition every stage of the process is visible in stained specimens and it is impossible to confuse these stages with multiple infection of the red corpuscle, if one has had experience in the examination of malarial blood. The photomicrographs which accompany this study show that in intracorpuscular conjugation we are not dealing with multiple infection of the erythrocyte.

Morphology of intracorpuscular conjugation.—This form of conjugation occurs only between two young hyaline forms of the plasmodia, indistinguishable in size and structure. The process, therefore, is isogamous in nature, the conjugants being undifferentiated. As the phenomena of this form of conjugation are similar in all of the species of human plasmodia, I shall describe it as observed in infections with Plasmodium falciparum, as it is very frequently observed in such infections.

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* NOTE.—I desire to express my thanks to Dr. William M. Gray, of the Army Medical Museum, for the photomicrographs which accompany this contribution. The magnification in all the photomicrographs, with the exception of Fig. 12, is ×1500. Figure 12 is ×1200. Photomicrographs 10, 11, and 12 are inserted for purposes of comparison with the pigmented conjugation form illustrated in Fig. 9.

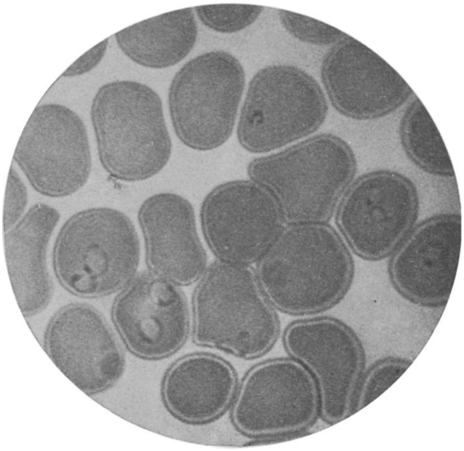

FIG. 2.—Estivo-autumnal infection. This specimen shows multiple infection of the erythrocyte and intracorpuscular conjugation, in which the conjugants are in the first and second stages of the process. Note the "rings" with the double dot of chromatin, and the two parasites which are in the first stage, before the complete union of the rings.

The process invariably occurs between two unpigmented "ring-forms" and is first evidenced by the union of the protoplasm of the two conjugants. Red corpuscles are observed containing two "ring-forms" lying in apposition and careful examination will demonstrate that at the point of contact there is undoubted union of the protoplasm of the two organisms. In fresh preparations the process may be followed in rare instances, and I have several times been so fortunate as to witness the complete union of the conjugants. In the fresh blood conjugation commences by the gradual approach of the two organisms until they are finally in apposition, the ameboid processes of the adjoining segments of the "rings" merging rapidly, so that in less than a moment there is no distinction between the two parasites at the point of apposition. A gradual blending of the protoplasm now occurs, until in a period varying from ten minutes to half an hour, all trace of two distinct organisms has been lost, and only one slightly larger plasmodium can be seen within the erythrocyte. In such preparations, of course, no structural details can be made out, but it is certain that while the two organisms concerned in conjugation become one in the time mentioned, the process is not completed, for, as shown in stained preparations, changes occur in the nucleus which require for their completion several hours. It is probable that intracorpuscular conjugation is not completed much within 24 hours.

In stained preparations it is usually observed that the primary union of the conjugating organisms occurs at some point remote from the nuclear chromatin. The latter may be situated at any portion of the plasmodia but in most instances the chromatin mass is situated on the outer periphery of the two "ring-forms" opposite the point of apposition. With the Wright stain the protoplasm of the plasmodia stains the usual blue color, the chromatin mass a brilliant crimson, while the vesicular portion of the nucleus remains unstained. Not infrequently "ring-forms" are observed in which the chromatin masses are apparently united but I do not consider such forms as conjugating organisms. They are independent plasmodia which are accidentally in contact in this manner. Careful examination will demonstrate that there is no union of the chromatin or of the protoplasm. That the apposition of the conjugants results in a true union of protoplasm is proven by the fact that there is no evidence of the outline of the "rings" at this point, or of overlapping. The chromatin masses are distinct and alike in appearance and the outline of the "rings" is preserved except at the point of contact (see Figs. E, 3, and 5).

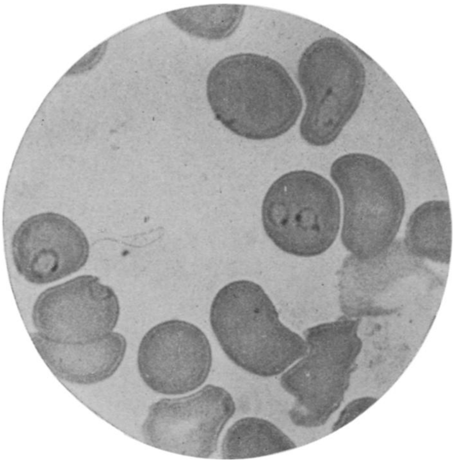

FIG. 3.—Estivo-autumnal infection. Showing multiple infection of the erythrocyte and one conjugation form which is distinguished by its large size and two masses of chromatin. In this organism protoplasmic union is complete and the resulting "ring" is twice as large as those in the doubly infected cell.

The initial stage of conjugation just described is followed by complete protoplasmic union, the two chromatin masses, in stained preparations, being observed within a single organism. While various examples of this stage of conjugation are observed in stained specimens, in all of them the slightly larger organisms resulting from the union of the two "rings" preserve their "ring" shape, the distinctive features being the increase in size and the presence within the organism of two distinct chromatin masses of equal size, and surrounded by an unstained area due to achromatic substance. The chromatin masses vary considerably in situation, sometimes being opposite one another, near the periphery of the organism, or lying almost in contact. All of the stages of union of the protoplasm as observed in stained specimens tend to show that the union is accompanied by a gradual absorption of the central portion of the adjoining segments of the "rings" until only a fine thread of protoplasm remains which eventually is absorbed and we have the production of the single, but larger "ring-shaped" plasmodium (see Fig. F).

The process of intracorpuscular conjugation is completed by the apparent union of the nuclei of the two plasmodia, which is evidenced in stained specimens by the union of the chromatin masses, accompanied by apparent reduction phenomena, and the extrusion of minute granules of chromatin. In such preparations plasmodia are observed illustrating various phases in the union of the chromatin masses and there can be no doubt that this union is marked by changes in the appearance of the chromatin and in its amount, while very frequently small dots of this substance are observed lying outside of the organism but within the protoplasm of the erythrocyte (see Fig. 7).

In many specimens it will be observed that the chromatin in one of the masses, instead of being collected in a compact mass, as it is in the other, is separated into very fine filaments or granules, and distributed over a segment of the "ring," thus bringing it nearer to the other mass of chromatin. This is apparently the first stage of nuclear union, and is observed only when the two chromatin masses have been situated opposite one another or some distance apart. Sometimes, under such circumstances, both masses of chromatin are broken up into filaments or granules (see Figs. I, 6).

In those instances in which the chromatin masses have been situated near one another, union appears to be effected by a lengthening of the masses until they meet, while in the first instance mentioned, the thinned mass of chromatin apparently travels around the intervening segments of the "ring" until it comes in contact with the other mass and unites with it (see Figs. J and K).

After the union of the two masses of chromatin, which generally

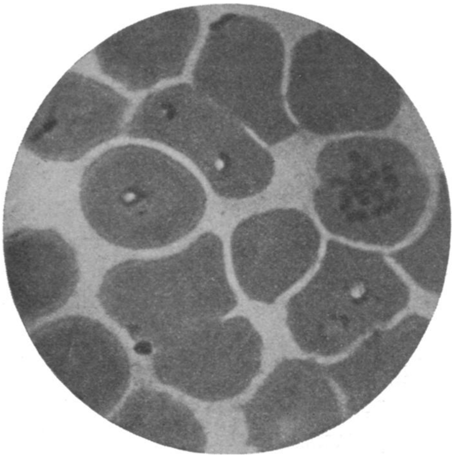

FIG. 4.—Estivo-autumnal infection. Showing various forms of conjugating organisms and a sporulating parasite. It will be seen that the chromatin dots in some of these organisms are almost united, while in others they have united, giving rise to a peculiar cupped appearance of this portion of the nucleus.

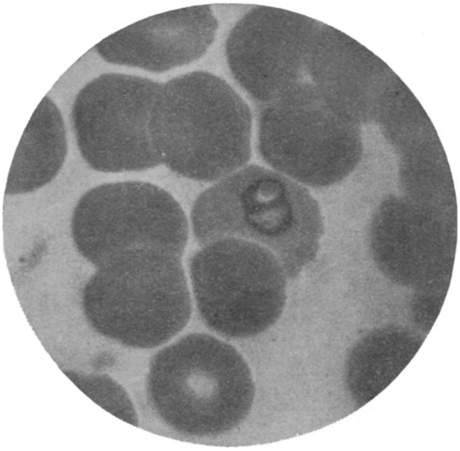

FIG. 5.—Tertian infection. Conjugating parasites in which protoplasmic union is almost complete, and the portions in apposition are beginning to be absorbed. The two chromatin masses are somewhat indistinct but are still separated.

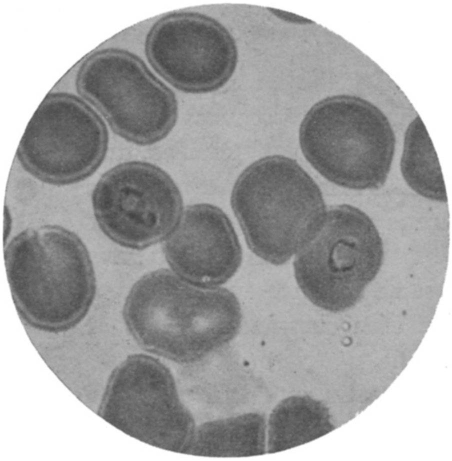

FIG. 6.—Tertian infection. Two conjugating forms in which the process is almost completed. Note the character of the chromatin masses.

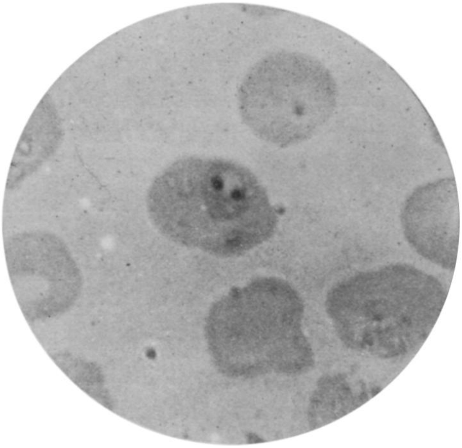

FIG. 7.—Tertian infection. A conjugating form in which the two chromatin masses are distinct and the portions of protoplasm in apposition have become absorbed, resulting in the formation of a large ring-like body with two chromatin masses. A small mass of extruded chromatin is seen lying in the infected cell.

occurs at some portion of the periphery of the organism, the chromatin takes up a position within the "ring," generally at the center, and is surrounded by an achromatic zone. When conjugation is complete the appearance of the chromatin is that of a compact, circular mass, staining a deep red, and of considerable size. The entire organism resembles very markedly the first stage in the growth of the malarial gametes, save that the organism is much larger and the chromatin mass is more deeply stained and of twice the size of the chromatin mass in the gametes.

In all stained specimens of blood from cases in which the malarial plasmodia have undergone schizogony for several generations, the stages of conjugation described can be observed, and it is evident that the process consists in a union of the protoplasm of the conjugants, followed by the union of the nuclei, accompanied by the extrusion of a small portion of the nuclear chromatin.

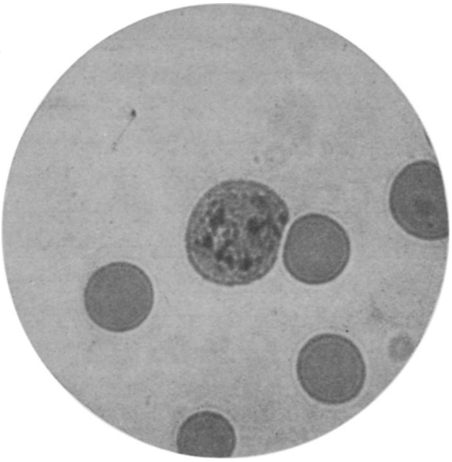

FIG. 8.—A large pigmented form resulting from conjugation. Tertian infection. This body is extracellular, has five large clumps of chromatin, and a very small amount of pigment. It is typical of the large pigmented forms which result from conjugation, and entirely distinct from the presegmenting form of the tertian plasmodium which is illustrated in Fig. 12.

It is not necessary to describe in full this process as it occurs in tertian and quartan infections, as it does not differ in any particular from the same process in the estivo-autumnal infections. Conjugation is completed during the hyaline stage before the formation of pigment, in both types of infection, but it is not always confined to the "ring-forms," for not infrequently conjugating forms are observed in which the active ameboid motions of the parasites have distorted the "rings," causing irregularly shaped organisms.

After he completion of conjugation the single parasite resulting develops within the erythrocyte and finally destroys it, becoming free in the blood. These developing parasites are distinguished from other forms of the plasmodia by the smaller amount of pigment, and, in stained specimens, by the collection of the chromatin in two or more irregular, deeply stained masses, distributed about the center of the organism (Figs. 8, N, O, and P).

In some instances the chromatin masses are numerous and are collected at one side of the organism, giving rise to an appearance suggesting sporulation. Such forms are very suggestive of the parthenogenetic gametes as pictured by Schaudinn in the contribution I have already referred to (see Fig. 9). The large pigmented forms are observed lying free in the blood plasma very frequently when conjugation is present but they never sporulate, nor have I ever observed exflagellation in any of these bodies. To my mind such forms as are illustrated in Fig. 9 are preparing for sporulation and are probably the forms which give rise to the young parasites which are responsible for recurrences, but they are certainly not macrogametes undergoing parthenogenesis, as they occur only *when conjugation is present,* and very frequently *before* gametes have appeared in the blood. In the estivo-autumnal infections these large pigmented forms are only observed in the blood obtained by splenic puncture, and they are not observed in any form of malarial infection in which intracorpuscular conjugation is absent. The growth of the parasite resulting from conjugation is rapid while it is contained within the erythrocyte but after being liberated there are no evidences of further growth and it is probable that the parasite then enters upon a resting stage, which may last for days or weeks, according to the conditions present.

It is these pigmented bodies which I believe were considered by Schaudinn to be macrogametes undergoing parthenogenesis, as his description of the presegmenting form of the macrogamete answers as well for the pigmented stages of the conjugating plasmodia. That this form of conjugation is of significance in the life-cycle of the plasmodia is evident from the occurrence of the process only in malarial infections which have existed for some time; the very uniform phenomena observed; the occurrence of developing forms dissimilar from other forms of the malarial plasmodia in the blood of patients showing conjugation; and the resemblance of the process to conjugation in other of the protozoa. The fact that typical conjugating

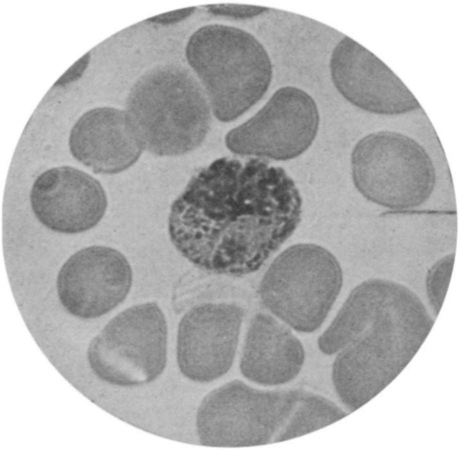

FIG. 9.—Tertian infection. Large pigmented form, the result of conjugation. This is the form which may have been considered by Schaudinn as a macrogamete undergoing parthenogenesis. Note the appearance resembling sporulation, the chromatin being divided into small masses and collected upon one side of the organism. These forms are very rare in the peripheral blood.

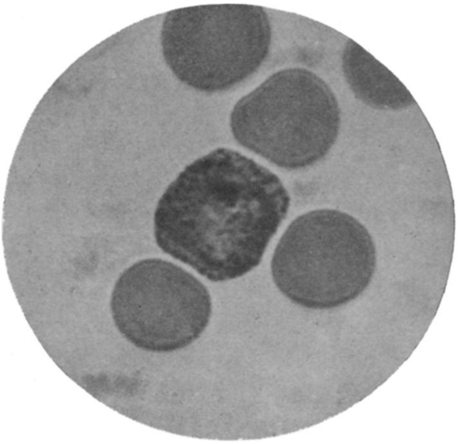

FIG. 10.—Tertian infection. A tertian macrogamete. Note large size, dense mass of chromatin, and large number of coarse granules of pigment.

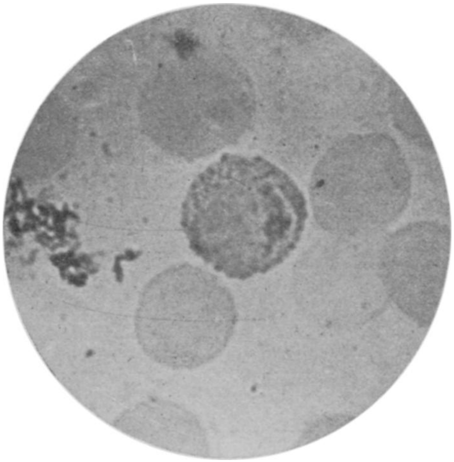

FIG. 11.—A tertian microgametocyte. Note the character of the chromatin mass as compared with that of the macrogamete; the finely granular pigment, which is also smaller in amount, and the poor staining quality of the parasite.

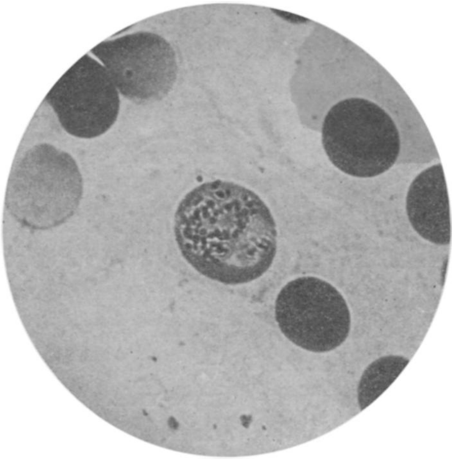

FIG. 12.—A typical presegmenting tertian plasmodium. Note the numerous small masses of chromatin and their distribution, as compared with the conjugation form shown in Fig. 8.

plasmodia are observed in the blood when all other hyaline forms have been replaced by pigmented forms, proves that the process has nothing to do with multiple infection of the erythrocyte, and that it requires several hours for its completion. For instance, in a typical single tertian infection, while all of the plasmodia undergoing schizogony have reached the presegmenting or three-quarter-grown stage, the hyaline conjugating bodies may also be present, and these are the only hyaline plasmodia observed. The presence of these forms in cases in which nearly all of the plasmodia have become pigmented often leads to the diagnosis of a double infection. So far as I have been able to observe neither the conjugating plasmodia, nor the large pigmented parasites developing from them, are affected by quinine. At least the changes which I have described as occurring in the schizogonic forms in Part I of this contribution I have never observed in the conjugating organisms.

Significance of the process of intracorpuscular conjugation.—I shall not, in this contribution, discuss the significance of conjugation in the Protozoa in general, which I have considered elsewhere,[1] but will simply state that the process is of great importance in the life-history of the organisms in which it has been observed. While the process of intracorpuscular conjugation in the malarial plasmodia has no exact analogue in other closely related parasites, it is but reasonable to believe that it is of importance in the life-history of the species of plasmodia in which it occurs. The morphological changes observed during the process of intracorpuscular conjugation are similar to those observed during the conjugation of other protozoan organisms and are of such a nature that they cannot be explained on the theory that they are plastogamic in nature, or accidental in occurrence. While plastogamy is very common in the Protozoa, it is not followed, as in intracorpuscular conjugation, by the development of forms of the parasite different in morphology from those usually observed—forms which are observed only after this process has occurred. The conjugating plasmodia do not separate after a period of time, but, as I have observed in fresh preparations, gradually blend and become one organism, which finally develops into the large pigmented body described. It is evident that the malarial plasmodia must exist in some form within man during the latent period of infection and between recurrences. I have previously shown that in latent infections (infections without symptoms) the plasmodia undergo normal schizogony within the spleen, but, while this is so, it is impossible to believe that this process can go on for weeks, and even months, without multitudes of plasmodia being produced, and, consequently, symptoms of the disease.

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[1] *Jour. Infect. Dis.*, 1907, 4, p. 108; *The Malarial Fevers, Haemoglobinuric Fever, and the Blood Protozoa of Man*, New York, 1909.

Various theories have been adduced to explain the occurrence of relapses in malaria, after long periods of time, and the phenomenon of latent malarial infection. Bignami considered that the plasmodia existed somewhere in the body in a latent form, perhaps encapsulated and spore-like in character, which, when conditions were favorable, multiplied and produced the forms usually observed in malarial infection. Celli believed that forms resulting from sexual multiplication remain inert in the viscera, such as the spleen and bone-marrow, and, when conditions were favorable, invaded the blood and gave rise to normal schizogonic forms. The observations of Schaudinn[1] are of especial interest in the consideration of this question. In his study of Plasmodium vivax he concluded that recurrences in malaria are due to parthenogenesis of the macrogametes which are not fertilized by the microgametes; these, remaining in man, eventually liberate schizonts which penetrate the erythrocytes, undergo sporulation, and produce the relapse. Schaudinn observed this process only in one case and describes it as consisting in the division of the chromatin of the nucleus, the death of a portion of the nucleus and protoplasm, and the segmentation of the remainder into forms which undergo schizogony. The forms undergoing parthenogenesis resemble very closely the forms resulting from intracorpuscular conjugation, so far as I have been able to follow them, and I believe that there is good reason for believing that at least some of the forms described by Schaudinn were in truth the large pigmented forms which result from this process.

Schaudinn claimed that parthenogenesis was complete in from nine to twelve days, and authorities, such as Ziemann and Mariotti-Bianchi, call attention to the occurrence of relaspes most frequently at this period of time. While relapses often do occur at intervals of nine to twelve days, it has not been my experience that the majority do, and it is very difficult, upon Schaudinn's assumption, to explain the occurrence of relapses occurring all the way from nine to eighty days after the initial attack, and the occurrence of latent infections, in which for weeks a few plasmodia may be demonstrated in the peripheral blood at intervals. I have observed relapses occurring at intervals of from two to four weeks very frequently, and several cases in which two to three months intervened between the malarial attacks. Taking these facts into consideration, I believe that it is much more rational to consider that the cause of relapse is some form of the parasite which is resistant to quinine, and which remains latent in the spleen or bone-marrow until conditions are favorable for development, when it undergoes mutiplication, and eventually develops into the forms which are familiar to us as schizonts; and I believe that it is the function of intracorpuscular conjugation to produce such forms of the plasmodia.

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[1] *Loc. cit.*

As is well known, rapid multiplication of certain protozoa leads to exhaustion and it is at this time in the life-history of the organisms that conjugation occurs, followed by a resting stage in some parasites, and subsequent multiplication when conditions are favorable for growth. It is significant that intracorpuscular conjugation occurs only in malarial patients who have suffered from repeated paroxysms; in other words, only in those cases in which rapid multiplication of the plasmodia has occurred for some time, in the usual manner. It is also more frequent in those cases in which quinine has been given in small and inefficient doses, and this again indicates that the process is one that develops only when conditions arise which are unfavorable to development in the normal manner. That the forms concerned in this process are resistant to quinine is another proof that they are intended to keep the infection alive within man.

The discontinuance of quinine in malarial patients is often followed by recurrences, even in patients who have been under treatment for a long time, and it is evident that during this time the forms of the plasmodia present in the body must have been resistant to the drug. Now, as a matter of fact, the only forms of the malarial plasmodia which resist quinine are the fully developed gametes and the forms concerned in intracorpuscular conjugation, and while the parthenogenesis of the macrogamete might explain the occurrence of such relapses, *if macrogametes were always present*, it is a fact that relapses occur in many patients in whom the initial infection has not resulted in the development of any form of gamete. Thus, in a very large proportion of estivo-autumnal patients, *relapses occurred repeatedly but the gametes (crescents) were never observed in either the peripheral blood or in blood obtained by splenic puncture.* In such cases, which were numerous, how can parthenogenesis of the macrogametes explain the occurrence of the relapses? Intracorpuscular conjugation is observed very frequently in patients in whom no gametes can be demonstrated, and these facts are sufficient proof, to my mind, that latency and recurrence in the malarial fevers cannot be explained by the parthenogenesis of the macrogamete, and that in intracorpuscular conjugation we probably possess the true explanation of these phenomena.

After a certain period of rapid multiplication, or when the environment becomes unfavorable for schizogony in the usual manner, the process of intracorpuscular conjugation occurs, resulting in the development of a form of the plasmodium which is resistant to quinine and other injurious influences, and which, in all probability, becomes encysted and retires to the spleen or bone-marrow, where it remains in a resting condition until the environment again becomes favorable, when it liberates the spores which have developed within it, and these, penetrating the erythrocytes, develop eventually into schizonts. While it must be confessed that all of the steps in this process have not been witnessed, it is a fact that every step has been demonstrated except the morphology of the organism in the resting stage in the spleen or bone-marrow, and the actual sporulation of the large pigmented bodies resulting from the conjugation. Further research, especially in pernicious malarial infections, should result in additions to our knowledge of the exact form in which the plasmodia exist in the internal organs, and will, I am sure, support the theory that intracorpuscular conjugation is the cause of latency and recurrence.

The administration of quinine to a patient in whom this form of conjugation is occurring, is followed by the disappearance of the conjugating plasmodia from the peripheral blood, but this does not prove that they are destroyed, but rather that they are driven by the presence of the drug to the internal organs. If the drug be administered before conjugation has begun, this process is never observed, and this is a strong argument in favor of the theory that this form of conjugation is the cause of recurrence, for it is well known that initial attacks of malaria, thoroughly and properly treated with quinine, do not suffer from relapses; while those that have been treated improperly, or in whom the infection has been present for several days before treatment, almost invariably relapse sooner or later.

In conclusion I would say that the importance of the subject of intracorpuscular conjugation, aside from its purely biological interest, lies in its relation to prophylaxis, for it is evident that if these forms of the plasmodia are the cause of relapse, it is essential that treatment be instituted promptly, and therefore, that it is necessary that the infection be recognized as early as possible. The early recognition of malarial infection depends upon the microscopical examination of the blood and this should never be neglected in any patient suffering from fever. The prophylaxis of malaria, especially in regions in which these infections are comparatively rare, often rests entirely in the hands of the attending physician, for the early recognition of the disease and the prompt institution of proper treatment will effectually put a stop to its spread, so far as that patient is concerned; while a false diagnosis, or improper treatment, will result in the patient becoming a source of infection to the community in which he resides.

EXPLANATION OF FIGURES A TO P.

The figures are diagrammatic and are intended to illustrate the phases of intracorpuscular conjugation observed in the blood in infections with Plasmodium vivax.

FIG. A.—Diagram of red blood corpuscle containing a "ring-form" of Plasmodium vivax: *A*, Chromatin of the nucleus of the plasmodium; *B*, Achromatic substance of the nucleus; *C*, Protoplasm of the plasmodium; *D*, Protoplasm of the red blood corpuscle.

FIG. B.—False conjugation. The two "ring-forms" are in apposition, the point of contact involving the chromatin only.

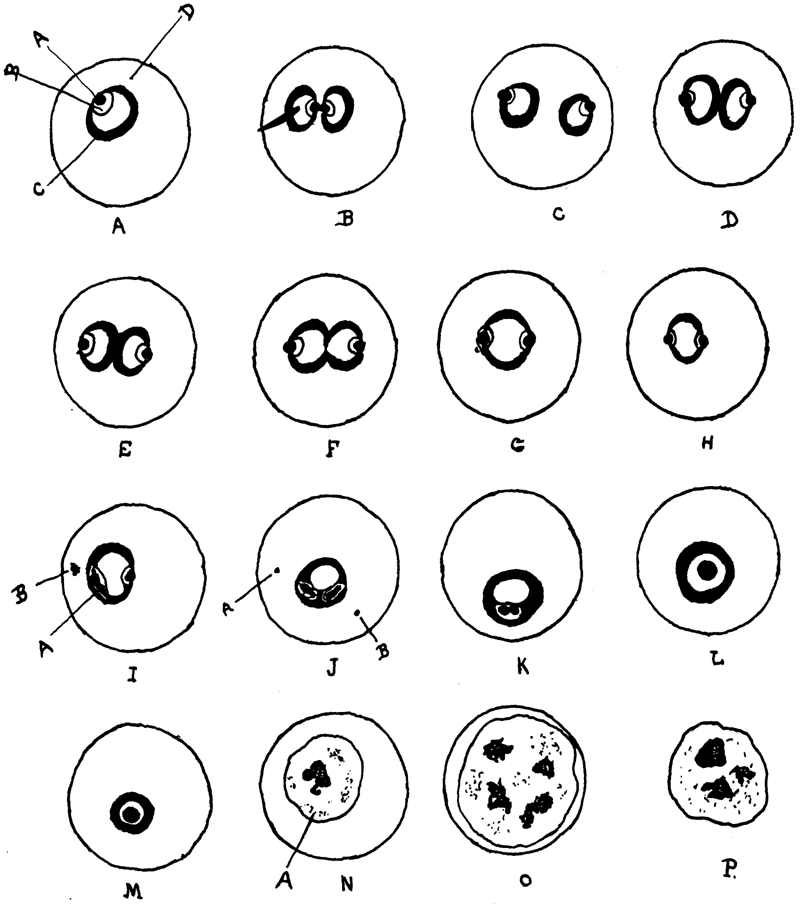
FIG. C.—Red blood corpuscle containing two "ring-forms" of Plasmodium vivax. No evidence of conjugation.

FIG. D.—First stage of conjugation. The "ring-forms" are in contact, the protoplasm alone being involved.

FIGS. E and F.—Illustrating the gradual blending of the protoplasm of the two organisms.

FIG. G.—Complete protoplasmic union of the two parasites. By this union one "ring-form" results, containing two masses of chromatin.

FIG. H.—Same as Fig. G but showing a smaller parasite.



FIGS. A–P.

FIG. I.—Conjugating organism in which the chromatin at *A* is lengthening and approaching the second mass of chromatin. At *B* a small particle of chromatin is seen which has been extruded from the conjugating organism.

FIG. J.—Conjugation form showing the approach of the two masses of nuclear chromatin. Note the lengthening of the chromatin masses. At *A* and *B* are observed extruded particles of chromatin.

FIG. K.—Conjugation form showing the union of the two chromatin masses, so that the organism now consists of one large "ring-form," containing a single large chromatin mass.

FIGS. L and M.—Forms resulting after conjugation is completed. The organism consists of a mass of protoplasm, containing a large chromatin mass surrounded by considerable achromatic substance. These forms, except for their large size, resemble closely the tertian gametes in their earliest stages.

FIGS. N, O, and P.—The large pigmented forms which develop from the conjugating forms. Note the small amount of pigment (*A*).